Journal of Food Protection, Vol. 60, No. 5, 1997, Pages 466–470

Factors Associated with the Presence of *Escherichia coli* O157 in Feces of Feedlot Cattle

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(MS# 96-142: Received 4 June 1996/Accepted 14 August 1996)

ABSTRACT

Fecal samples were collected from pens of cattle in a total of 100 feedlots in 13 states. Fecal samples were cultured for *Escherichia coli* O157. *E. coli* O157 isolates were probed for the genetic coding for verotoxin production. At the time of sample collection, data were collected on the type of cattle present in the pen, as well as the length of time these cattle were in the feedlot, ingredients for the current ration, and cattle health history since arriving in the feedlot. Factors associated with increased likelihood of a pen being positive (one or more samples probe-positive for *E. coli* O157) included feeding of barley (odds ratio [OR] = 2.75) and cattle being on feed less than 20 days (OR = 3.39). Factors associated with a reduced likelihood of a pen being positive included feeding soy meal (OR = 0.50), a cattle entry weight of at least 700 lb (ca. 317.5 kg) (OR = 0.54), and at least 85% of the cattle in the pen being beef-type heifers (OR = .33).

Key words: *Escherichia coli* O157, risk factors, shedding, feedlot, cattle

An outbreak of human disease associated with the consumption of undercooked hamburger in 1993 has focused intense interest on food-borne diseases. The causative agent of this outbreak, which occurred in the western United States, was identified as *Escherichia coli* O157:H7 (ECO157) *(2, 5)*. In this outbreak, several children developed hemolytic uremic syndrome, resulting in four deaths *(5)*. Increased public awareness and the desire to decrease the risk of food-borne illness has led to educational campaigns, regulations regarding labeling of meat products, regulations requiring hazard analysis and critical control point programs for slaughter plants, and increased research into the ecology of potential food-borne pathogens across the food-production continuum.

Previous studies have evaluated the frequency of recovery of ECO157 from the feces of cattle (Table 1). The animal or fecal sample prevalence has varied from 0.0% to 2.2% depending upon the type of cattle and geographic location of the study. As knowledge of the ECO157 organism has expanded, researchers have begun to focus on *E. coli* that have genetic coding for the production of verotoxin (ECO157VT) (thought to be important in the pathogenesis of human disease) rather than on the particular type of somatic or flagellar antigen present (in this case, O157:H7) *(14)*. Hancock et al. *(8)* reported that the frequency of recovery of ECO157VT from feedlot cattle feces was relatively low (1.8% of samples). However, 63% of the feedlots participating in the study had at least 1 positive fecal sample. In order to identify potential critical control points for ECO157VT in meat production, more information is needed at various points along the production continuum. At the farm level, further elucidation of management factors influencing the shedding of ECO157VT by cattle and other animals is needed. The objective of this study was to identify management factors associated with the presence of ECO157VT in the feces of feedlot cattle.

MATERIALS AND METHODS

Fecal sampling

The source of the fecal samples for this study has been described in greater detail elsewhere *(8)*. A convenience sample of 100 feedlots with at least 1,000 head one-time capacity that were participating in the United States Department of Agriculture, Animal and Public Health Inspection Service (USDA, APHIS), Veterinary Services, Cattle on Feed Evaluation was selected to submit fecal samples to be evaluated for ECO157VT presence. Four cattle pens in each feedlot were selected for sampling on the basis of time on feed (two pens) and random selection (two pens). The pens with cattle that had been on feed the shortest (short-fed) and longest (long-fed) amount of time (one of each) were always selected. In addition, the remaining pens were numbered sequentially and two pens (if available) were selected for sampling using a random numbers table. Within each pen, 30 fresh moist fecal pats from the pen floor were swabbed. Efforts to collect samples from various locations throughout the pens were made to minimize the probability of collecting multiple swabs from feces of the same animal.

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TABLE 1. *Sample and animal prevalence of ECO157 from previous studies*

Period (year)	Number and type of animals, premises	Prevalence of ECO157 in: Animal	Herd	Fecal sample	Reference
1986	226 dairy cattle 2 premises	2.2%	100%		*(13)*
	428 dairy cattle 11 premises	1.2%	27.3%		*(13)*
	46 dairy cattle 1 stockyard	2.2%	—		*(13)*
1987	539 dairy cattle 9 premises	1.3%	55.5%		*(13)*
	27 dairy heifers and calves 1 packing house	0.0%	—		*(13)*
1991	3,570 dairy cattle 60 premises	0.3%	8.3%		*(7)*
1992	1,412 beef cows 25 premises	0.7%	16.0%		*(7)*
1991–1992	600 feeder cattle 5 feedlots	0.3%	40.0%		*(7)*
1991–1992	6,894 dairy calves 1,068 premises	0.4%	1.8%		*(9)*
1994	11,881 samples 100 feedlots		63.0%	1.61%	*(8)*

Laboratory methods

Two laboratories received samples via overnight mail. The sample processing used in this study has been described elsewhere *(8)*. Sample-processing methods for this study were different between the two laboratories because of individual laboratory constraints. Samples for laboratory 1 were transported in Cary-Blair medium *(1)* while Trypticase soy broth (Difco Laboratories, Detroit, MI) with vancomycin (Lyphomed, Deerfield, IL) and cefixime (Lederle, Pearl River, NY) was used for transport of samples to laboratory 2. In addition, samples submitted to laboratory 1 were held for an average of 3.6 days (range of 0 to 10) under refrigeration prior to being placed into the enrichment medium, modified EC broth with novobiocin *(10)*. Samples submitted to laboratory 2 were held for 1 day or less prior to being placed in the enrichment medium.

Data collection

Questionnaires were administered in person to feedlot operators by trained interviewers. Questions addressed general management practices used in each feedlot and specific attributes of the pens where fecal samples were collected (the type and number of cattle present, duration of time on feed, general health, and nutritional management factors such as components of the current diet). Dietary component questions included the type of forage, concentrate, protein supplement, feed additives (e.g., ionophores, antibiotics, or probiotics), and any other ingredients (e.g., by-products or tallow).

Data analysis

Feedlot pens were classified as positive if ECO157VT was recovered from at least 1 fecal swab per pen. The pen status (positive or negative) was the outcome of interest for this analysis. Several categorical variables were defined on the basis of the characteristics of the cattle and management practices in the pens (Table 2). Previous research has indicated that a period of nutritional deprivation followed by feeding may increase the shedding of ECO157VT in the feces of cattle *(4)*. This type of nutritional stress can occur in cattle recently placed in the feedlot. For this reason, two types of pens were defined, those on feed for fewer than 20 days and those on feed for 20 or more days.

All categorical variables were screened for association with pen-level ECO157VT status by use of a chi-square test using statistical software *(11)*. Variables associated with the outcome ($P \leq 0.25$) were candidates for inclusion in a multiple logistic

TABLE 2. *Categorical variables and definitions screened for association with pen-level ECO157VT*

Variable[a]	Definition
HFRPEN	At least 85% of the animals in the pen were beef-type heifers
STRPEN	At least 85% of the animals in the pen were beef-type steers
DRYPEN	At least 85% of the animals in the pen were dairy-type
COWPEN	At least 85% of the animals in the pen were beef-type cows
BULLPEN	At least 85% of the animals in the pen were beef-type bulls
DYFEDCAT ..	Animals on feed less than 20 days
HLTH	General health of the pen of cattle (below normal, normal, above normal)
ENT700	Animal at least 700 lb on entry
DENCAT	At least 220 ft^2 (ca. 20–44 m^2) per animal
NLAB	Laboratory where sample testing done
AMPR	Amprolium in the current diet
DECO	Decoquinate in the current diet
IONO	Ionophore in the current diet
MOLA	Molasses in the current diet
PROB	Probiotics in the current diet
TETR	Tetracycline in the current diet
OANT	Antibiotic other than tetracycline in the current diet
UREA	Urea in the current diet
YEAS	Yeast in the current diet
BARL	Barley in the current diet
BRGM	Brewer's grains/malt in the current diet
CORN	Corn in the current diet
GSOR	Grain sorghum (milo) in the current diet
WHEA	Wheat in the current diet
WHFN	Wheat fines in the current diet
BEET	Beet pulp in the current diet
CANO	Canola meal in the current diet
WCTS	Whole cotton seed in the current diet
CTSM	Cotton seed meal in the current diet
MEAT	Meat and bone meal in the current diet
SOYM	Soybean meal in the current diet
ALFA	Alfalfa hay/haylage in the current diet
CLOV	Clover hay/haylage in the current diet
CSIL	Corn silage in the current diet
CTSH	Cotton seed hulls in the current diet
SILA	Forage sorghum hay/silage in the current diet
TALL	Tallow in the current diet
BYPR	By-products in the current diet

[a] All variables were dichotomous with the exception of HLTH, which had three levels.

regression model. The initial multiple logistic regression model was constructed using a backward elimination algorithm. To remain in the model, variables had to be associated with pen-level ECO157VT shedding with $P \leq 0.05$. Since data from multiple pens were collected from each feedlot (up to four per feedlot), there was potential confounding by feedlot-level attributes in the data. To adjust for this, the data were transferred to SUDAAN statistical software *(12)* where feedlot clustering was controlled for explicitly. SUDAAN allows adjustment for within-group clustering, as explicitly specified in estimation of variance by first forming the Taylor series linearization for each statistic, which are then substituted into the formula for computing the variance appropriate for the design specified by the user.

RESULTS

Samples positive for ECO157VT were identified in 63 of the 100 participating feedlots. Of the 398 pens sampled, 101 (25.4%) had 1 or more positive samples. In most instances, 30 samples per pen were collected and tested (Table 3). The number of positive samples per pen ranged from 0 to 10 (Table 4). Status of the pen (positive or negative) was associated ($P \leq 0.25$) with 18 variables describing nutritional management, animal demographics, or testing laboratory, by using chi-square analysis (Table 5). Using a backward elimination algorithm to remove variables from the model, only six variables remained in the multiple logistic regression model (Table 6). When the final model was run using SUDAAN, as expected there was essentially no change in the estimates of the beta coefficients after controlling for clustering by feedlot (Table 7). There was also little change in the standard errors of the beta coefficients after controlling for clustering by feedlot (Table 7).

When the model controlled for laboratory and other management variables simultaneously, pens with cattle that were currently receiving barley in the ration were 2.75 times more likely to have a positive sample compared to pens with cattle that were not currently receiving barley in the diet (Table 7). Pens of cattle that had been on feed for less than 20 days were 3.39 times more likely to have a positive sample than pens of those on feed for longer periods of time. The likelihood of pens having a sample test positive was lower for those composed of at least 85% heifers (odds ratio [OR] = 0.33), with average entry weights of at least 700 pounds (OR = 0.54) and receiving soybean meal (OR = 0.50). The association of receiving soybean meal with pen status was marginally significant ($P = 0.06$) after controlling for clustering of data by feedlot.

There was no statistical association between shedding status of the cattle in a pen and the current feeding of

TABLE 3. *Numbers of samples collected and tested per pen*

No. pens	No. samples per pen
1	13
1	14
2	20
6	29
388	30

TABLE 4. *Number of pens by number of positive samples per pen*

No. positive samples per pen	No. pens
0	297
1	53
2	21
3	15
4	7
5	0
6	1
7	0
8	2
9	1
10	1

TABLE 5. *Variables associated with recovery of ECO157VT from fecal swabs from feedlot pens*

		Pens			*P* value
Variable	Levels	No. positive	No. negative	% positive	(chi square)
IONO	Yes	78	246	24.1	.211
	No	23	51	31.1	
UREA	Yes	66	216	23.4	.159
	No	35	81	30.2	
BARL	Yes	18	22	45.0	.003
	No	83	275	23.2	
CORN	Yes	88	275	24.2	.094
	No	13	22	37.1	
GSOR	Yes	20	43	31.7	.205
	No	81	254	24.2	
WHEA	Yes	6	9	40.0	.185
	No	95	288	24.8	
WCTS	Yes	9	12	42.9	.059
	No	92	285	24.4	
CTSM	Yes	29	61	32.2	.090
	No	72	236	23.4	
SOYM	Yes	20	90	18.2	.041
	No	81	207	28.1	
CSIL	Yes	40	149	21.2	.066
	No	61	148	29.2	
HLTH	Below average	3	15	16.7	.185
	Average	62	152	29.0	
	Above average	36	130	21.7	
HFRPEN	Yes	18	105	14.6	.001
	No	83	192	30.2	
STRPEN	Yes	59	152	28.0	.208
	No	42	145	22.5	
DRYPEN	Yes	12	24	33.3	.250
	No	89	273	24.6	
DYFEDCAT	<20 days	44	62	41.5	<.001
	≥20 days	57	235	19.5	
ENT700	<700 lb	64	146	30.5	.013
	≥700 lb	37	151	19.7	
DENCAT	<220 ft²/animal	55	140	28.2	.204
	≥220 ft²/animal	46	157	22.7	
NLAB	lab 1	51	188	21.3	.023
	lab 2	50	109	31.4	

TABLE 6. *Model parameters for the multiple logistic regression model without accounting for clustering by feedlot*

Variable	Definition	Coefficient	SE	OR[a]	95% CI OR[b]
INTRCPT		0.10	.29		
BARL	Feeding barley	1.00	.37	2.73	1.32–5.61
SOYM	Feeding soy meal	−0.69	.31	0.50	0.27–0.92
HFRPEN	≥85% beef type heifers	−1.11	.31	0.33	0.18–0.61
NLAB	Lab 2	0.63	.25	1.88	1.15–3.06
DYFEDCAT	On feed <20 days	1.22	.27	3.39	2.00–5.75
ENT700	Entry wt ≥ 700 pounds	−0.62	.25	0.54	0.33–0.88

[a] Odds of recovering ECO157VT from pen.
[b] 95% confidence interval for the odds ratio.

TABLE 7. *Model parameters after accounting for clustering by feedlot*

Variable	Definition	Coefficient	SE	OR[a]	95% CI OR[b]
INTRCPT		1.91	.60		
BARL	Feeding barley	1.01	.30	2.75	1.53–4.94
SOYM	Feeding soy meal	−0.69	.36	0.50	0.25–1.02
HFRPEN	≥85% beef type heifers	−1.11	.32	0.33	0.18–0.62
NLAB	Lab 2	0.63	.25	1.88	1.15–3.06
DYFEDCAT	On feed <20 days	1.22	.26	3.39	2.03–5.64
ENT700	Entry wt ≥ 700 pounds	−0.62	.26	0.54	0.32–0.90

[a] Odds of recovering ECO157VT from pen.
[b] 95% confidence interval for the odds ratio.

antibiotics, coccidiostats, ionophores, probiotics, urea, or other feed additives evaluated. General health of the pen of cattle while in the feedlot was not associated with pen shedding status. Animal density in pens was not associated with shedding status of cattle in those pens.

DISCUSSION

Other studies have shown associations of ECO157 fecal shedding in cattle with nutritional and management variables *(3, 6, 9)*. In one study *(9)*, an association was demonstrated between routine use of ionophores in the feed of dairy heifers and increased prevalence of ECO157. In a subsequent study *(6)*, a similar association between shedding and ionophore feeding was not found. In this study, there was no indication of an association between ionophore use and outcome status after controlling for other attributes of the pens of cattle. Because the large majority of feedlots fed an ionophore to cattle in the randomly selected pens (85% of randomly selected pens) and in the pens that had been on-feed the longest (89% of long-fed pens), there was a concern about the lack of statistical power to detect an association between ionophore feeding and pen status. A subgroup analysis was limited to the pens of short-fed cattle. Of the 65 pens of short-fed cattle that were receiving an ionophore at the time of sampling, 40% were ECO157VT positive. These differences were not statistically significant ($P = 0.78$), nor were there significant differences ($P = 0.68$) in the average days on feed for the pens of short-fed cattle receiving an ionophore (6.6 days) and pens of short-fed cattle not receiving an ionophore (7.7 days). In addition, there was no indication of an association between the use of antibiotics in feedlot cattle rations and recovery of ECO157VT from pen fecal samples.

An association between the gender of cattle and ECO157VT shedding status has not been demonstrated previously. The lower likelihood of recovering ECO157VT from pens with at least 85% heifers may be related to specific management of heifers compared to management of steers or may be a spurious finding. More controlled trials may be able to identify the source of this association.

The use of barley in the current diet of cattle was associated with an increased likelihood of recovering ECO157VT from fecal samples. The association of barley feeding with positive fecal samples may be related to digestion dynamics, including gastrointestinal transit times or fermentation patterns (site and speed). Barley feeding has been considered to be strongly regional in practice and as such the association of barley feeding with pens being positive could reflect a regional distribution of positive pens rather than a link with barley feeding. In this study, barley was fed in 40 pens of cattle in 11 different feedlots. These feedlots were located in Arizona, California, Idaho, Texas, and Washington. Positive pens that fed barley were found in each of the five states. More work will be required to define the basis for this observed relationship between barley feeding and ECO157VT.

Cattle receiving soybean meal in their current diet were less likely (by pen groups) to shed ECO157VT in their feces. However after accounting for the clustering of pens by feedlot, this association was not significant ($P = 0.06$) at the traditional level of statistical significance. At this point in our understanding of the ecology of this organism, a more liberal level of statistical significance may be justified. Similar associations were not present for nonprotein nitrogen sources or other natural protein sources (e.g., beet pulp, meat and bone meal, or cotton seed meal). More work should be done to evaluate the repeatability of this finding. If the finding is repeatable, efforts should be made to understand how this diet component can alter shedding of ECO157VT.

Pens of cattle on feed less than 20 days were 3.4 times more likely to have a positive sample. This may be the result of the short-term feed deprivation that can occur during the transit process prior to arrival at the feedlot or other stresses on animals newly arrived in the feedlot. Garber et al. *(6)* demonstrated that grouping dairy heifers on the dairy operation was associated with increased shedding of ECO157. Wells et al. *(13)* and Zhao et al. *(15)* have indicated that shedding of ECO157 can be intermittent. Perhaps stresses induce periodic shedding and there are periods of nonshedding between stress episodes. Yet another explanation is that commingling of cattle could result in previously naive cattle becoming colonized with ECO157VT and subsequently shedding at detectable levels. Perhaps shedding levels then decrease to a point where detection using current methods is not possible. It is also possible that some earlier feeding regimen prior to arrival in the feedlot is affecting the

shedding status of cattle in the first 20 days after entry to the feedlot. Once again, more controlled studies will be necessary to evaluate each of these hypotheses.

Pens of cattle with entry weights of 700 pounds or more were less likely to have positive samples even after adjusting for other variables like diet components and number of days on feed. Older, heavier animals may be more immunocompetent and better able to handle the stress of transportation and a new environment. Younger, lighter cattle may be more naive in terms of exposure to bacterial or viral organisms. Either mechanism could be responsible for the reduced shedding in pens of cattle that were heavier when they arrived at the feedlot.

The fact that the standard errors for the point estimates of the odds ratios changed very little when clustering was accounted for in the analysis seems to indicate that there was a lack of clustering of positive pens by feedlot. This seems reasonable, since ECO157VT is not generally considered an organism that would propagate through a population of cattle as in an outbreak. Also, knowing that cattle enter feedlots from many sources and reflect many herds, it seems reasonable that there would be nearly as much variation among cattle within a feedlot as among feedlots. It would not be surprising that exposure and colonization of these animals with specific organisms would show similar patterns.

The current study is the largest study of ECO157VT in feedlot cattle to date. The feedlots studied were in 13 states where in excess of 85% of the feedlot cattle population for the United States are located. The wide geographic distribution of these feedlots together with the variety of management attributes represented in the data set provides a broadly based reference population for the study results.

There are a number of limitations associated with the current study. First, shedding of ECO157VT at detectable levels has been shown to vary over time within an individual animal *(13, 15)*. The failure to find the agent in a pen of cattle on a particular day does not mean that the cattle within that pen were negative for their entire feeding period. Second, since fecal samples were collected from the pen floor, the sample prevalence does not necessarily reflect the animal prevalence. Although steps were taken to minimize the probability of collecting multiple fecal samples from an individual animal, such occurrences could not be ruled out. Likewise since multiple samples could have come from the same animal, the true power to detect any shedding in the pen may be somewhat less than calculated when assuming the samples came from a random sample of individual animals in the pen. Third, all of the feed-ingredient data represented current exposures (on the day of sample collection or in the previous seven days). No data were available on previous feed ingredients that animals may have been exposed to or the duration of exposure to current diet components. It is possible that a particular feed ingredient which is truly causally linked to an increased (or decreased) likelihood of shedding within the pen (if one existed) may not have been fed for a sufficient duration for the effect to be detected by the time samples were collected.

The goal of this study was to generate testable hypotheses on factors associated with ECO157VT shedding from a large population of cattle with external validity. New hypotheses regarding the ecology of ECO157VT in cattle and more specifically in feedlot cattle were generated from this study. It is also important to note that a number of factors were not associated with ECO157VT shedding in this study. Among these were ionophore use, feeding antibiotics, animal density within pens, previous pen-level health status of cattle, and coccidiostat use.

ACKNOWLEDGMENTS

The authors wish to thank all of the veterinary medical officers and animal health technicians involved in data and sample collection for this project. Also, thanks to the staff at the National Veterinary Services Laboratories and the Washington State University Field Disease Investigation Unit for their technical assistance in sample processing.

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